

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
19 April 2001 (19.04.2001)

PCT

(10) International Publication Number  
**WO 01/26481 A1**

- (51) International Patent Classification<sup>7</sup>: A23K 1/16, (74) Agent: MURGITROYD & COMPANY; 373 Scotland Street, Glasgow G5 8QA (GB).  
1/18, A61K 31/505, 31/52, A61P 1/00
- (21) International Application Number: PCT/GB00/03899 (81) Designated States (*national*): AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.
- (22) International Filing Date: 11 October 2000 (11.10.2000)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:  
9924096.2 13 October 1999 (13.10.1999) GB  
0009853.3 25 April 2000 (25.04.2000) GB
- (71) Applicant (*for all designated States except US*): EWOS LIMITED [GB/GB]; Ewos Technology Centre, Unit 1, Kingsthorpe Park, Houston Industrial Estate, Livingston EH54 5DB (GB).
- (72) Inventors; and
- (75) Inventors/Applicants (*for US only*): BURRELLS, Charles [GB/GB]; 3 Inkerman Court, Penicuik, Midlothian EH26 0LS (GB). WILLIAMS, Paul, David [GB/GB]; 12 Montrose Road, Auchterarder, Perth & Kinross PH3 1BZ (GB).
- (84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).
- Published:  
— With international search report.  
— Before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments.
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

(54) Title: FISH FEED WITH INCREASED NUCLEOTIDE CONTENT

(57) Abstract: The present invention provides for the improved uptake of feed additives, pigments for use in colouring the flesh and the like. This improved intestinal uptake of foodstuffs and additives is effected through enhancing the development of the intestinal tract. Specifically the present method comprises of feeding fish with a diet which has an augmented level of nucleotides present therein. The nucleotides may be selected from the group consisting of uracil, guanine, cytosine, thymine, adenine and mixtures thereof. The resultant improvement in the development of the intestinal tract leads to an improvement of the intestinal surfaces to absorb nutrients and/or dietary additives, this increase being at least partly due to an increase in gut surface area, for example through an increase in villus height.

WO 01/26481 A1

1 FISH FEED WITH INCREASED NUCLEOTIDE CONTENT

2

3 The present invention relates to a method of  
4 improving the development of the gut intestinal tract  
5 in fish. Specifically, by means of supplementing the  
6 normal levels of nucleotides present in the diet of  
7 the fish, improved differentiation and development of  
8 the cells of the intestinal tract occurs, this having  
9 the advantageous effect of enhancing intestinal  
10 absorbtion of feed additives and pigmentation for  
11 colouring the flesh.

12

13 It has been previously shown in the literature that  
14 dietary nucleotides are known to exert a significant  
15 influence on many mammalian immunological and  
16 physiological functions. More specifically, it is  
17 known that these nucleic acid precursors are  
18 instrumental in the healthy differentiation and  
19 development of the cells of the intestinal tract.  
20 Diets supplemented with nucleotides lead to increased

1 intestinal mass, gut wall thickness, accelerated  
2 mucosal repair after infection or diarrhoea and  
3 enhanced populations of gut micro flora. In  
4 mammalian species, it has been demonstrated that  
5 additional dietary nucleotides induce an increase in  
6 the height of the intestinal villi resulting in an  
7 overall expansion of the total mucosal surface area.  
8 These studies have, so far, been confined to  
9 mammalian species.

10

11 It is an object of the present invention to provide a  
12 method of enhancing the development of the intestinal  
13 tract in fish.

14

15 A further object of the present invention is to  
16 provide a method of facilitating an increase in the  
17 intestinal uptake of foodstuffs and additives.

18

19 According to the present invention there is provided  
20 a method of improving the development of the  
21 intestinal tract in fish, the method comprising  
22 augmenting the levels of nucleotides present in the  
23 diet of fish.

24

25 By "development of the intestinal tract" it is meant  
26 that the ability of the intestinal surfaces to absorb  
27 nutrients and / or dietary additives is increased.  
28 Generally, this increase in absorbtion is at least in  
29 part due to an increase in gut surface area, for  
30 example by an increase in villus height.

31

1 Advantageously, the nucleotides may be selected from  
2 the group consisting of uracil, guanine, cytosine,  
3 thymine, adenine and mixtures thereof.

4

5 The present invention encompasses the use of any  
6 nucleotide and the nucleotides may be comprised  
7 either of a single nucleotide type or as a mixture in  
8 combination with other nucleotide types.

9

10 It should be noted that modified (eg methylated),  
11 synthetically derived mimetics or functionally  
12 equivalent molecules of uracil, guanine, cytosine,  
13 thymine and adenine may be used in the present  
14 invention.

15

16 Preferably the dietary nucleotide level is augmented  
17 by means of providing either a dietary composition  
18 comprising high levels of nucleotides or by providing  
19 a feed in which nucleotide levels have been  
20 deliberately augmented.

21

22 In one embodiment, the present invention provides a  
23 dietary composition of the feed, which contains at  
24 least 0.01% of nucleotides and preferably at least  
25 0.02% of nucleotides, relative to the total weight of  
26 the diet.

27

28 Surprisingly it has been found that the increased  
29 nucleotide dietary concentration leads to enhanced  
30 levels of absorption of dietary pigment, consequently  
31 we have observed a change in flesh colour of the fish

1 fed nucleotide enhanced diets in combination with  
2 dietary pigments.

3

4 The invention also provides the use of nucleotides to  
5 enhance uptake of pigment by fish.

6

7 Accordingly, the present invention also provides a  
8 method of modifying fish flesh colour, said method  
9 comprising the dietary administration of nucleotides  
10 and pigment.

11

12 It is anticipated that the uptake of non-pigment  
13 dietary additives will likewise be enhanced. Dietary  
14 additives in question include medicaments such as  
15 antibiotics, vaccines, vitamins, and the like. Thus,  
16 the present invention provides a method of enhancing  
17 the intestinal uptake of such additives in fish by  
18 enhancing the nucleotide content of the diet.

19

20 In a further aspect, the present invention provides a  
21 dietary composition comprising nucleotide and pigment  
22 or other dietary additives. Suitable pigments  
23 include astaxanthin, and in one preferred embodiment  
24 the dietary composition comprises astaxanthin and  
25 0.028% by weight nucleotides.

26

27 Our research has been conducted using dietary  
28 supplements with high levels (about 14%) of the  
29 nucleotides. Diets would already contain levels of  
30 endogenous nucleotides, but a supplement added at  
31 0.2% would add about a further 0.03% of nucleotides.

1 Research has shown that supplementation for feeds  
2 induces similar changes in the morphology of the  
3 piscine intestinal tract to those found in other  
4 animals. Trials in fish of various weights ranging  
5 from below 40g to 2,000g indicated that the villus  
6 heights in the distal intestines of these fish were  
7 increased by up to 21.5%.

8  
9 Further experimentation investigating and quantifying  
10 the effects of the introduction of increased  
11 nucleotides into fish by means of inclusion in  
12 dietary intake are outlined below, in the following,  
13 non-limiting example.

14  
15 **Example 1**

16  
17 Fish of about 200g were fed a diet containing about  
18 0.2% of the supplement which contained about 14%  
19 nucleotides (diets therefore contained about 0.028%  
20 added nucleotides) for 3 weeks. At the end of this  
21 period villus height in the distal region of the gut  
22 were about 21% greater than in the fish fed control  
23 diets. Several similar trials have been carried out  
24 in different sizes of fish; in all cases similar  
25 results were achieved.

26  
27 Further to the finding that villus height was  
28 increased by the addition of supplementary levels of  
29 nucleotides, an additional effect of the in feed  
30 nucleotide was revealed by experimentation. It has  
31 been observed that fish fed a nucleotide supplemented

1 diet exhibited a greater pigment coloration in their  
2 flesh. It is hypothesised that the increased gut  
3 surface area which resulted due to the initial  
4 addition of the nucleotides in the diet, enhanced  
5 absorbtion of dietary pigment into the blood which in  
6 turn led to improved deposition of pigment in the  
7 muscle. This enhanced deposition effect may allow a  
8 reduction in the dietary pigment concentration for  
9 the desired flesh coloration of consequential  
10 implications for raw material costs.

11

## 12 **Example 2**

13

14 Fish of initial weight of about 100g were fed on  
15 diets including 0.2% of the supplement containing  
16 about 14% nucleotides (diet contained about 0.028% of  
17 nucleotides) for 17 weeks. Treated fish showed  
18 pigment (astaxanthin) levels of 9% or greater than  
19 seen in the control experiments.

20

21 It can be concluded that, due to the physiological  
22 effect induced by the addition of nucleotides to the  
23 diet and of increasing the villus size, there is a  
24 supplementary effect of enhancing the absorption and  
25 uptake of other feed additives.

26

27 At present an enhanced absorption of dietary pigment  
28 into the blood has been shown to lead to improved  
29 deposition of colouring pigment in the muscle.

30

1 This principle can be further extended to predict  
2 that the increased villus size and resultant increase  
3 in absorptive surface area of the intestine, due to  
4 increased levels of dietary nucleotides will also  
5 lead to enhanced uptake of other feed additives such  
6 as protein based additives, antigenic preparations or  
7 therapeutic substances including antibiotics.



## 1 CLAIMS

2

3 1. A method of improving the development of the  
4 intestinal tract in fish, the method comprising  
5 augmenting the levels of nucleotides present in the  
6 diet of fish by providing at least one compound  
7 chosen from nucleotides or a modified or  
8 synthetically derived mimetics or functionally  
9 equivalent molecules to nucleotides.

10

11 2. A method as claimed in claim 1, wherein the  
12 compound is selected from the group consisting of  
13 uracil, guanine, cytosine, thymine, adenine and  
14 mixtures thereof.

15

16 3. A method as claimed in claim 1 or claim 2  
17 wherein at least one nucleotide comprises either a  
18 single nucleotide type or a mixture in combination  
19 with other nucleotide types.

20

21 4. A method as claimed in any previous claim  
22 wherein modified or synthetically derived mimetics or  
23 functionally equivalent molecules of uracil, guanine,  
24 cytosine, thymine and adenine are used.

25

26 5. A fish feed which contains at least 0.01% of  
27 nucleotides relative to the total weight of the feed.

28

29 6. A fish feed which contains at least 0.02% of  
30 nucleotides, relative to the total weight of the  
31 diet.

1 7. The use of at least one nucleotide or a modified  
2 or synthetically derived mimetic or functionally  
3 equivalent molecule to enhance uptake of pigment by  
4 fish.

5  
6 8. A method of modifying fish flesh colour, said  
7 method comprising the dietary administration of at  
8 least one nucleotide or modified or synthetically  
9 derived mimetic or functionally equivalent molecule  
10 and pigment to fish.

11  
12 9. A method as claimed in claim 8 which further  
13 facilitates the uptake of non-pigment dietary  
14 additives.

15  
16 10. A method as claimed in claim 8 or claim 9  
17 wherein dietary additives include medicaments such as  
18 antibiotics, vaccines and vitamins.

19  
20 11. A method of enhancing the intestinal uptake of  
21 additives in fish by enhancing the nucleotide content  
22 of the diet.

23  
24 12. Use of nucleotides to enhance intestinal uptake  
25 of additives by fish.

26  
27 13. A dietary composition comprising nucleotide and  
28 pigment or other dietary additives.

29  
30 14. A composition as claimed in claim 13 wherein the  
31 pigment is astaxanthin.

1 15. A composition as claimed in claims 13 or 14  
2 wherein the dietary composition comprises astaxanthin  
3 and 0.028% by weight nucleotides.  
4

5 16. Use of nucleotides in the preparation of a  
6 medicament to increase intestinal uptake of  
7 medicaments by fish.  
8

9 17. Use of nucleotides in the preparation of a  
10 medicament for the treatment of fish.

## INTERNATIONAL SEARCH REPORT

International Application No

P/GB 00/03899

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 A23K1/16 A23K1/18 A61K31/505 A61K31/52 A61P1/00

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A23K A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, CHEM ABS Data, CAB Data, BIOSIS

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 776 490 A (CHU FU-LIN E ET AL) 7 July 1998 (1998-07-07) column 3, line 23 - line 27	13,16,17
Y	column 3, line 49 - line 56 column 4, line 41 - line 45 column 8, line 33 - line 57 examples 1,2 figure 5	1,11,12, 16,17
Y	WO 96 11707 A (OREGON STATE) 25 April 1996 (1996-04-25) page 6, line 25 - line 26 page 11, line 4 - line 8	1,11,12, 16,17
	--- -/- ---	

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

## \* Special categories of cited documents:

- \*A\* document defining the general state of the art which is not considered to be of particular relevance
- \*E\* earlier document but published on or after the international filing date
- \*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- \*O\* document referring to an oral disclosure, use, exhibition or other means
- \*P\* document published prior to the international filing date but later than the priority date claimed

- \*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- \*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- \*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- \*&\* document member of the same patent family

Date of the actual completion of the international search

30 January 2001

Date of mailing of the international search report

14/02/2001

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax (+31-70) 340-3016

Authorized officer

Date received

BEST AVAILABLE COPY

## INTERNATIONAL SEARCH REPORT

International Application No

P/GB 00/03899

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>DATABASE WPI  Section Ch, Week 199538  Derwent Publications Ltd., London, GB;  Class D13, AN 1995-287919  XP002158862  -&amp; JP 07 184595 A (NIPPON SEISHI KK),  25 July 1995 (1995-07-25)  abstract</p>	5,6,13
X	<p>US 5 188 851 A (MAMOTO KATSUHIRO ET AL)  23 February 1993 (1993-02-23)  column 2, line 33 - line 39  claims 1-8</p>	5,6,13
X	<p>FERNANDO KUBITZA ET AL.: "Identification  of feed enhancers for juvenile largemouth  bass Micropterus salmoides"  AQUACULTURE,  vol. 148, no. 2-3, 1997, pages 191-200,  XP000979269  AMSTERDAM, NL  page 191, abstract  page 194; table 2  page 198, paragraph 2</p>	5,6,13
X	<p>GARY L. RUMSEY ET AL.: "Nutritional value  of dietary nucleic acids and purine bases  to rainbow trout (Oncorhynchus mykiss)"  AQUACULTURE,  vol. 108, no. 1-2, 1992, pages 97-110,  XP000979268  AMSTERDAM, NL  the whole document</p>	5,6,13
X	<p>DATABASE WPI  Section Ch, Week 198209  Derwent Publications Ltd., London, GB;  Class D13, AN 1982-16704E  XP002158863  -&amp; JP 57 012972 A (TAKEDA CHEM IND LTD),  22 January 1982 (1982-01-22)  abstract</p>	13
A	<p>WO 97 40702 A (OLLEVIER FRANS ;ROELANTS  IVO (BE); LEUVEN K U RES &amp; DEV (BE); AGRO)  6 November 1997 (1997-11-06)  page 9, line 7 - line 28  page 10, line 32 - line 33  claims 1-8,17,32,33</p>	1,4-6, 11-13, 16,17

-/--

## INTERNATIONAL SEARCH REPORT

In tional Application No

P GB 00/03899

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>DATABASE WPI Section Ch, Week 198711 Derwent Publications Ltd., London, GB; Class B04, AN 1987-076355 XP002158864 &amp; JP 62 029530 A (NISSHIN FLOUR MILLING CO), 7 February 1987 (1987-02-07) abstract</p> <hr/>	13

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 00/03899

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
US 5776490	A	07-07-1998	NONE	
WO 9611707	A	25-04-1996	US 5871751 A AU 4000195 A CA 2202499 A GB 2308300 A, B NO 971650 A	16-02-1999 06-05-1996 25-04-1996 25-06-1997 06-06-1997
JP 7184595	A	25-07-1995	JP 2756907 B	25-05-1998
US 5188851	A	23-02-1993	JP 1776101 C JP 3164140 A JP 4067942 B DK 275890 A NO 176992 B	28-07-1993 16-07-1991 29-10-1992 22-05-1991 27-03-1995
JP 57012972	A	22-01-1982	NONE	
WO 9740702	A	06-11-1997	AU 2893097 A EP 0923312 A	19-11-1997 23-06-1999
JP 62029530	A	07-02-1987	JP 1784353 C JP 4073416 B	31-08-1993 20-11-1992